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Biosystematic Studies on the Family Tofieldiaceae II. Phylogeny of Species of *Tofieldia* and *Triantha* Inferred from Plastid and Nuclear DNA Sequences

MINORU N. TAMURA[1*], HIROSHI AZUMA[1], JUN YAMASHITA[2],
SHIZUKA FUSE[3] AND TAKAAKI ISHII[4]

[1]*Department of Botany, Graduate School of Science, Kyoto University, Kitashirakawa-oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan. *mntamura@sys.bot.kyoto-u.ac.jp (author for correspondence);* [2]*Research Institute for Bioresources, Okayama University, 2-20-1 Chuo, Kurashiki-shi, Okayama 710-0046, Japan;* [3]*Museum of Nature and Human Activities, Hyogo, 6 Yayoigaoka, Sanda-shi, Hyogo 669-1546, Japan;* [4]*Botanical Gardens, Graduate School of Science, Osaka City University, 2000 Kisaichi, Katano-shi, Osaka 576-0004, Japan*

Using plastid *trnK*, *trnL* and *trnL–F* sequences and nuclear ITS sequences (4558 bp in total) from 32 individuals of 13 species, we conducted a combined analysis to clarify phylogenetic relationships of the species of *Tofieldia* and *Triantha* and to discover new taxonomic characters. According to strict consensus of 45 most parsimonious trees, the two genera are monophyletic with 100% bootstrap support. In *Tofieldia*, three robust clades (100%) are formed: (1) *To. coccinea, To. nuda, To. divergens, To. yunnanensis* and *To. thibetica*, (2) *To. calyculata*, and (3) *To. okuboi, To. glabra* and *To. pusilla*. The species of the clade 3 are primarily characterized by a broad and sharply bent leaf apex as in some species of *Triantha.* In clade 1, the Chinese species of *To. divergens, To. yunnanensis* and *To. thibetica* are grouped (100%). They share long needle-like or hair-like processes on the leaf margin. In *Triantha*, two clades are formed: (1) *Tr. japonica, Tr. occidentalis* and *Tr. glutinosa* from the USA (76%), and (2) *Tr. racemosa* and *Tr. glutinosa* from Canada (91%). This might suggest that *Tr. glutinosa* is the assemblage retaining ancestral character states of *Triantha* and should be divided at least into two species in the future treatments.

Key words: Alismatales, ITS, molecular phylogeny, taxonomic characters, *Tofieldia*, Tofieldiaceae, *Triantha*, *trnK*, *trnL*, *trnL–F*

Tofieldiaceae (Alismatales) are rhizomatous perennial herbs formerly included in Liliaceae. The species grow in rocky alpine places, alpine meadows, marshes, savannas, rock faces along rivers, etc., and often on calcareous soils. The flowers are actinomorphic, homochlamydeous, trimerous, hypogynous, and nearly apocarpous. The capsules dehisce septicidally. These character states are believed to be ancestral in the monocotyledons, and Tofieldiaceae are often considered to be one of the morphologically most primitive families among the monocotyledons (Thorne 1992 for Melanthiaceae, Takhtajan 1997, Tamura 1998 for Nartheciaceae).

The only molecular phylogeny of Tofieldiaceae (Tamura *et al.* 2004a) revealed that Tofieldiaceae consist of *Tofieldia* Huds., *Triantha* (Nutt.) Baker, *Harperocallis* McDaniel and *Pleea* Michx., and resolved the phylogenetic relationships among the four genera. Since that paper aimed at circumscribing Tofieldiaceae, many of the species were not included. Except for the monotypic *Harperocallis* and *Pleea*, only five species of *Tofieldia* and only two species of *Triantha* were analyzed.

In this study, we focus on the *Tofieldia* and *Triantha*. *Triantha* differs from *Tofieldia* in usually having three flowers per node, glandular hairs, and appendaged seeds. Nevertheless, *Triantha* has often been included in *Tofieldia*. For example, *Triantha japonica* (Miq.) Baker, endemic to Japan, was treated as *Tofieldia* in all published Japanese floras (e.g. Ohwi 1953, Kitamura 1964, Satake 1982). Tamura *et al.* (2004a) showed that the clade of *Triantha* is independent and sister to the clade containing the species of *Tofieldia*. The results, however, were based on an analysis of only several species.

We recognize 11 species of *Tofieldia*. *Tofieldia coccinea* Richardson and *To. pusilla* (Michx.) Pers. are distributed widely in boreal regions. The other species, including *To. divergens* Bureau & Franch., *To. glabra* Nutt., *To. nuda* Maxim., *To. okuboi* Makino and *To. thibetica* Franch., are localized. In spite of this interesting contrast in distribution ranges, the relationships of the species of *Tofieldia* have never been resolved.

Triantha comprises four species: *Tr. glutinosa* (Michx.) Baker, *Tr. occidentalis* (S. Watson) R. R. Gates and *Tr. racemosa* (Walter) Small in North America (Packer 2002), and *Tr. japonica* in Japan (Tamura in press). *Triantha glutinosa* is widely distributed in the USA and Canada, while the other three species are narrowly localized. Also in *Triantha*, the phylogeny of the species has never been analyzed by molecular methods.

The first aim of this paper is to clarify the phylogenetic relationships of the species of *Tofieldia* and *Triantha* using ample materials of the two genera, although we were unable to analyze the Russian *Tofieldia cernua* Sm. and the Himalayan *Tofieldia himalaica* Baker. To achieve our goals, we sequenced both plastid and nuclear DNA regions, which were 4558 bp in total after the alignment.

Perhaps because of the simple morphology of Tofieldiaceae, very few morphological characters have been used for the taxonomy of *Tofieldia* and *Triantha*. The second aim of this paper is to discover new taxonomic characters for *Tofieldia* and *Triantha* by analyzing the detailed phylogenetic relationships of species of the two genera based on the molecular analysis.

Materials and Methods

Plant materials

DNA sequences of the plastid regions of the *trnK* gene (including *matK*), *trnL* gene and *trnL–F* intergenic spacer and the nuclear region of ITS for nine species, one subspecies and one variety (20 OTUs) of *Tofieldia*, three species (10 OTUs) of *Triantha*, and *Harperocallis flava* McDaniel and *Pleea tenuifolia* Michx. (as the outgroup) were determined (Table 1). We selected the outgroup based on the results of Tamura *et al.* (2004a). As for *Triantha occidentalis* subsp. *occidentalis* and *Tr. occidentalis* subsp. *brevistyla* (C. L. Hitchc.) Packer, we failed in determining the DNA sequences of *trnK* and ITS, and thus we obtained data for only *trnL* and *trnL–F* (Table 1). All of the sequence data are deposited in the DNA Data Bank of Japan (DDBJ) database.

DNA extraction, PCR amplification and DNA sequencing

Total genomic DNA was extracted from fresh leaves, silica gel dried leaves, or leaves of herbarium specimens from the Missouri Botanical Garden (MO) and Kyoto University (KYO), by the slightly modified cetyltrimethylammonium bromide (CTAB) method of Doyle & Doyle (1987).

The *trnK*, *trnL* plus *trnL–F*, and ITS regions were amplified using the polymerase chain reaction (PCR) with a programmable temperature-control system GeneAmp PCR System 9600 or 2700 (Applied Biosystems). The amplification reaction mixture was prepared using TaKaRa Ex Taq DNA polymerase following its instruction manual (Takara Shuzo). We used sixteen, two, and six PCR primers (Table 2) for the amplification of *trnK*, *trnL* + *trnL–F*, and ITS regions, respectively, and the following PCR profile: a 30–37 cycle reaction with denaturation at 94°C for 0.5–1 min, annealing at 50–62°C for 0.5–1 min, and extension at 72°C for 0.5–3 min, in addition to an initial denaturation at 94°C for 2.5–5 min and a final extension at 72°C for 5–7 min. We purified the PCR products with MicroSpin S-300 HR Columns (Pharmacia Biotech) or QIAquick

TABLE 1. Sources of materials of Tofieldiaceae

Taxon	Locality	Voucher	Acc. no. (*trnK*)	Acc. no. (*trnL* & *trnL-F*)	Acc. no. (ITS)
Tofieldia calyculata Wahlb.	Germany: Frankfurt, Palmengarten	*C. Bayer s.n.* (KYO)	AB541027	AB541059	AB541078
	Germany: Garmisch-Partenkirchen, Schachen, 1700 m	*A. Gröger 20.07.03* (KYO)	AB541028	AB541060	AB541079
	Italy: Lago di Garda, Val di Vesta, 550 m	*Vleminckx F. 643* (MO)	AB541029	AB451579	AB541080
	Italy: Prov. de Trento, Tremalzo, 1800 m	*P. Maquet 82/90* (MO)	AB541030	AB451580	AB541081
Tofieldia coccinea Richardson					
var. *coccinea*	USA: Alaska, Lime Hills, 550–670 m	*C. L. Parker & R. Lipkin 8884* (MO)	AB541031	AB451586	AB541082
var. *kondoi* (Miyabe & Kudo) H. Hara	Japan: Hokkaido, Mt. Apoi, 635 m	*M. N. Tamura & S. Fuse 15227* (KYO)	AB541032	AB541061	AB541083
	Japan: Nagano Pref., Mt. Amakazari, 1950 m	*M. N. Tamura, S. Fuse & T. Ishii 14344* (KYO)	AB541033	AB541062	AB541084
Tofieldia divergens Bureau & Franch.	China: Yunnan, Dali, 2530 m	*M. N. Tamura 4704* (KYO)	AB541034	AB541063	AB541085
Tofieldia glabra Nutt.	USA: North Carolina, S of Jacksonville	*H. N. Moldenke 113* (MO)	AB541035	AB451590	AB541086
Tofieldia nuda Maxim.	Japan: Mie Pref., Washiyama, 110 m	*M. N. Tamura, S. Fuse & T. Ishii 15171* (KYO)	AB541036	AB541064	AB541087
	Japan: Hyogo Pref., Kamegatsubo, 430 m	*M. N. Tamura & S. Fuse 15532* (KYO)	AB541037	AB541065	AB541088
Tofieldia okuboi Makino	Japan: Hokkaido, Mt. Yubari, 1480 m	*M. N. Tamura & S. Fuse 15220* (KYO)	AB541038	AB541066	AB541089
	Japan: Hokkaido, Mt. Yubari, 1355 m	*M. N. Tamura & S. Fuse 15216* (KYO)	AB541039	AB541067	AB541090
	Japan: Hokkaido, Mt. Yubari, 1345 m	*M. N. Tamura & S. Fuse 15215* (KYO)	AB541040	AB541068	AB541091
	Japan: Ishikawa Pref., Mt. Hakusan, 2580 m	*M. N. Tamura & S. Fuse 15519* (KYO)	AB541041	AB541069	AB541092
	Japan: Ishikawa Pref., Mt. Hakusan, 2500 m	*M. N. Tamura & S. Fuse 15521* (KYO)	AB541042	AB541070	AB541093
Tofieldia pusilla (Michx.) Pers.					
subsp. *pusilla*	Denmark: Greenland, SW of Norujuk, 5 m	*B. Fredskild & C. Bay 3148* (MO)	AB541043	AB451593	AB541094
subsp. *austriaca* H. Kunz	Austria: Mt. Dachstein, 2030 m	*M. N. Tamura & S. Fuse 16074* (KYO)	AB541044	AB541071	AB541095
Tofieldia thibetica Franch.	China: Sichuan, Hongya, 1150 m	*C. H. Li 394* (MO)	AB541045	AB451596	AB541096
Tofieldia yunnanensis Franch.	China: Yunnan, Kunming, 1980 m	*S. X. Yuan 256* (MO)	AB541046	AB451589	AB541097
Triantha glutinosa (Michx.) Baker	USA: Alaska, Indian River Valley, 40 m	*C. L. Parker 5190* (MO)	AB541047	AB451599	AB541098
	Canada: Alberta, Banff National Park, 1450 m	*F. J. Breteler 13914* (MO)	AB541048	AB451597	AB541099
	USA: Idaho, Custer	*Summers & al. 9354* (MO)	AB541049	AB451598	AB541100
Triantha japonica (Miq.) Baker	Japan: Yamagata Pref., Mt. Chokai, 1650 m	*M. N. Tamura & S. Fuse 10135* (KYO)	AB541050	AB541072	AB541101
	Japan: Ishikawa Pref., Mt. Hakusan, 2100 m	*M. N. Tamura & S. Fuse 15528* (KYO)	AB541051	AB541073	AB541102
	Japan: Ishikawa Pref., Mt. Shakadake, 1800–1900 m	*H. Okada, Syamsuardi & Tesri M. 5511* (OCU*)	AB541052	AB541074	AB541103
	Japan: Shiga Pref., Mt. Mikuni, 800 m	*M. N. Tamura 13801* (KYO)	AB541053	AB541075	AB541104
Triantha occidentalis (S. Watson) R. R. Gates					
subsp. *occidentalis*	USA: California, NE of Stirling, 1750 m	*L. Ahart & P. Ahart 4758* (MO)	———**	AB451601	———**
subsp. *brevistyla* (C. L. Hitchc.) Packer	USA: Washington State, Snoqualmie Nat. Forest, 2150 m	*J. W. Thompson 15079* (MO)	———**	AB451602	———**
Triantha racemosa (Walter) Small	USA: South Carolina, Santee Coastal Reserve	*S. R. Hill 23888* (MO)	AB541054	AB451604	AB541105
	USA: Alabama, Perdido	*M. Groves s.n. 8-98* (GA)	AB541055	AB541076	AB541106
	USA: Florida, Walton	*W. S. Judd 2261* (MO)	AB541056	AB451603	AB541107
Harperocallis flava McDaniel	USA: Florida, Liberty	*S. B. Jones 25193* (MO)	AB541057	AB451606	AB541108
Pleea tenuifolia Michx.	USA: North Carolina, Wilmington	*F. H. Utech 78-300* (KYO)	AB541058	AB541077	AB541109

* OCU: herbarium, Botanical Gardens, Graduate School of Science, Osaka City University.

** DNA sequences of the *trnK* and ITS regions of *Triantha occidentalis* subsp. *occidentalis* and *T. occidentalis* subsp. *brevistyla* were unable to be determined.

PCR Purification Kit (Qiagen).

Direct sequencing was conducted with 25, 7, and 7 primers (Table 2) for *trnK*, *trnL* + *trnL–F*, and ITS regions, respectively, using an ABI PRISM 310 or 3100 Genetic Analyzer (Applied Biosystems) and a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems), according to the manufacturer's instructions. Mostly we sequenced both strands, but in a few cases we sequenced only a single strand. We manually aligned the DNA sequences obtained.

Phylogenetic analysis

We employed the maximum parsimony method for phylogeny reconstruction using PAUP* version 4.0 beta 10 (Swofford 2002). Heuristic search option of random addition sequence (100 replications), tree bisection and reconnection (TBR) swapping algorithm and multiple parsimonious trees (MULPARS) were used to find the most parsimonious trees. Only the base substitutions were treated as characters for phylogenetic reconstruction. All base substitutions were equally weighted. The gaps were treated as missing values. The consistency index (CI), retention index (RI), and rescaled consistency index (RC) were calculated with PAUP*, including uninformative characters. Bootstrap analysis with 1000 replications (heuristic; 100 random; TBR) was performed using PAUP*.

Congruence among the data sets of three plastid regions, *trnK*, *trnL* and *trnL–F*, and also congruence among the combined data set of the three plastid regions and the nuclear ITS data set were examined with the incongruence length difference (ILD) test (Farris *et al.* 1994), implemented in PAUP*, as the partition homogeneity test. The tests were performed with 100 replications of heuristic search with random addition sequence (100 replications), TBR swapping, and MULPARS.

Results and Discussion

DNA sequence variation

In *Tofieldia*, the plastid *trnK* genes ranged from 2536 to 2643 bp, the plastid *trnL* genes from 544 to 579 bp, the plastid *trnL–F* intergenic spacers from 234 to 250 bp, and the nuclear ITS regions from 574 to 589 bp. Values of pairwise sequence divergence ranged from 0.0 to 3.8% for *trnK*, to 5.1% for *trnL*, to 5.6% for *trnL–F*, and to 8.7% for ITS.

DNA sequences of *Triantha* were much less variable than those of *Tofieldia*. In *Triantha*, the *trnK* genes ranged from 2605 to 2627 bp, and the ITS regions from 573 to 575 bp. The *trnL* genes were a constant 554 bp in all OTUs of *Triantha*. The *trnL–F* intergenic spacers were also consistently 241 bp in length in all OTUs. Values of pairwise sequence divergence ranged from 0.0 to 2.3% for *trnK*, to 1.2% for *trnL*, to 0.8% for *trnL–F*, and to 3.0% for ITS.

Phylogenetic analysis

Combining all OTUs analyzed in this study into the same data set, of the 2986 bp of *trnK* sequences that were aligned for the analysis, 563 bp (18.9%) were variable, and 307 bp (10.3%) were phylogenetically informative. Of the 618 bp of aligned *trnL* sequences, 77 bp (12.5%) were variable, and 49 bp (7.9%) were phylogenetically informative. Of the 295 bp of aligned *trnL–F* sequences, 51 bp (17.3%) were variable, and 22 bp (7.5%) were phylogenetically informative. Of the 659 bp of aligned ITS sequences, 291 bp (44.2%) were variable, and 152 bp (23.1%) were phylogenetically informative.

The partition-homogeneity test among the data sets of three plastid regions, *trnK*, *trnL* and *trnL–F*, found a P value of 0.66. Considering the incongruence threshold of 0.05, we concluded that the data sets of the three plastid regions were combinable. Further, the partition-homogeneity test of the combined data set of the three plastid regions and the nuclear ITS data set found a P value of 0.17. Thus, we concluded that the data set of three plastid regions and the nuclear ITS data set were also combinable.

The combined analysis of the plastid *trnK*, *trnL* and *trnL–F* sequences and nuclear ITS sequences resulted in 45 equally most parsimonious trees with 1371 steps. The CI was 0.85, the RI 0.93, and the RC 0.78.

TABLE 2. Name, direction, DNA sequence and reference for PCR and sequence primers used in this study

Primer name	Direction	DNA sequence (5′–3′)	Reference
[Plastid: *trnK* gene (including *matK*)]			
trnK-11[a]	Sense	CTCAACGGTAGAGTACTCG	Liston & Kadereit (1995)
trnK-300F[a]	Sense	GGGTTTAGTGAATAAATGGATAG	Yamashita & Tamura (2000)
trnK-314F[a]	Sense	AATGGATAGAGTCCTATGGCCCC	Newly designed
trnK-400R[a]	Antisense	AACCTTTTCCCGCATCAGGC	Yamashita & Tamura (2000)
trnK-407R[b]	Antisense	CACAGAGGAACCCTTTCCCGC	Newly designed
trnK-450F[a]	Sense	GATTATGGAGCGTAGGCAGA	Newly designed
trnK-650R[a]	Antisense	CCACTAAACAGGGAGTCCAATC	Newly designed
trnK-697F[a]	Sense	CCTTGTTCTGACCATATCGCAC	Newly designed
trnK-706R[a]	Antisense	ATTATACATAGTGCAATATGGTC	Yamashita & Tamura (2000)
matK-750F[b]	Sense	GATCTCGGCAACAACGCTTC	Newly designed
matK-800R[b]	Antisense	GAGTAACGGATATAAGAAGCGTTG	Newly designed
matK-AF[b]	Sense	CTATATCCACTTATCTTTCAGGAGT	Ooi *et al.* (1995)
matK-850R[b]	Antisense	CCTAAAATTTCCATGGGTTCGTG	Newly designed
matK-1080F[b]	Sense	AAGTTTTGCAATTATTGTAG	Yamashita & Tamura (2000)
matK-1150F[a]	Sense	GAAATACCAAAATCTCAGAA	Fuse & Tamura (2000)
matK-1235F[b]	Sense	TATACTAATACCCCACCCC	Johnson & Soltis (1995)
matK-1235R[a]	Antisense	GGGGTGGGGTATTAGTATA	Johnson & Soltis (1995)
matK-1350F[a]	Sense	TACAATGTCGGATACAAGATGTTCC	Newly designed
matK-1470R[a]	Antisense	AAGATGTTGATCGTAAATGA	Johnson & Soltis (1995)
matK-1649F[b]	Sense	TCTTTTGATGAAGAAATGGA	Tamura *et al.* (2004b)
matK-1700R[b]	Antisense	ACCAAAAGTGAAAATAATATTGCCA	Tamura *et al.* (2004b)
matK-1900F[a]	Sense	CATCCCATTAGTAAGCCGAT	Newly designed
matK-8F[a]	Sense	TCGACTTTCTTGTGCTAGAACTTT	Steele & Vilgalys (1994)
matK-8R[a]	Antisense	AAAGTTCTAGCACAAGAAAGTCGA	Steele & Vilgalys (1994)
trnK-2621[a]	Antisense	AACTAGTCGGATGGAGTAG	Liston & Kadereit (1995)
[Plastid: *trnL* gene & *trnL–F* intergenic spacer]			
trnL-c[a]	Sense	CGAAATCGGTAGACGCTACG	Taberlet *et al.* (1991)
trnL-400F[b]	Sense	CACTCCAGAGTCTGATTGAT	Newly designed
trnL-400R[b]	Antisense	ATCAATCAGACTCTGGAGTG	Newly designed
trnL-420F[b]	Sense	CAAGAATAAAGAGAGAGTCC	Newly designed
trnL-d[b]	Antisense	GGGGATAGAGGGACTTGAAC	Taberlet *et al.* (1991)
trnL-e[b]	Sense	GGTTCAAGTCCCTCTATCCC	Taberlet *et al.* (1991)
trnF-f[a]	Antisense	ATTTGAACTGGTGACACGAG	Taberlet *et al.* (1991)
[Nuclear: ITS]			
18S-F[a]	Sense	AAGTTCGTAACAAGGTTTCCGTAGGTG	Bogler *et al.* (1995)
ITS-7F[b]	Sense	GGTTTCCGTAGGTGAACCTGCGGAAGGATC	Newly designed
ITS-8F[a]	Sense	GGTGGCGGTCCAATGAACAAACCCC	Newly designed
ITS3[a]	Sense	GCATCGATGAAGAACGCAGC	White *et al.* (1990)
ITS2[a]	Antisense	GCTGCGTTCTTCATCGATGC	White *et al.* (1990)
ITS-9R[a]	Antisense	GCTCATGTCCACCACTCATCGTGACTTGTC	Newly designed
ITS4[a]	Antisense	TCCTCCGCTTATTGATATGC	White *et al.* (1990)

[a] Used for both PCR amplification and DNA sequencing

[b] Used for only DNA sequencing

According to the strict consensus tree (Fig. 1), the species of *Tofieldia* are grouped with 100% bootstrap support, and the species of *Triantha* are also grouped (100%). This independence between *Tofieldia* and *Triantha*, which was already indicated in Tamura *et al.* (2004a) based on a molecular phylogenetic analysis, is substantiated using an ample sample size in this study. These findings are consistent with recent taxonomic treatments in which *Triantha* is often regarded as distinct from *Tofieldia* (Utech 2002, Tamura in press).

In *Tofieldia*, three robust clades (100%) were formed. Clade 1 comprises *To. coccinea*, *To. nuda*, *To. divergens*, *To. yunnanensis* Franch. and *To. thibetica*; clade 2 contains *To. calyculata* Wahlb., and clade 3 includes *To. okuboi*, *To. glabra* and *To. pusilla*. The species of clade 3 are characterized by the broad leaf apex being sharply bent as in some species of *Triantha*, although the leaf apex of *To. pusilla* is sometimes gently curved (Fig. 2). The leaf apex of the other species of *Tofieldia* analyzed in this study is more or less slender, and straight, gently curved or slightly bent (Fig. 2). Leaf apex morphology has been infrequently used as a taxonomic character in *Tofieldia*, but this study revealed that the morphology of the leaf apex is of great taxonomic value in *Tofieldia*.

In the clade of *Tofieldia coccinea*, *To. nuda*, *To. divergens*, *To. yunnanensis* and *To. thibetica*, two subclades (100%) were further formed: one of the circumboreal *To. coccinea* and the eastern Asian *To. nuda*, and the other of the remaining three species. The latter three species, all endemic to China, share long needle-like (Fig. 3A) or hair-like (Fig. 3B & C) processes on the leaf margin. Scabrous versus smooth leaf margins are sometimes used as a diagnostic character in *Tofieldia*. In addition, however, the shape and size of the processes on the leaf margin may also be valuable in the taxonomy of *Tofieldia* (Fig. 3). Among the three Chinese species, *To. divergens* and *To. yunnanensis* are grouped (100%). This is not contradictory to the treatment of Chen & Tamura (2000) in which *To. yunnanensis* is reduced to *To. divergens*. In the clade of *To. okuboi*, *To. glabra* and *To. pusilla*, two subspecies of *To. pusilla*, subspp. *pusilla* and *austriaca* H. Kunz, are grouped (100%) as expected, while *To. okuboi* from Japan and *To. glabra* in the eastern USA are also grouped (89%).

In *Triantha*, two clades were formed. One clade (76%) included *Tr. japonica*, *Tr. occidentalis* and *Tr. glutinosa* from Alaska and Idaho, USA, and the other clade (91%) contained *Tr. racemosa* and *Tr. glutinosa* from Alberta, Canada. The results may suggest that *Tr. glutinosa*, whose distribution is much wider than the distribution of the other three species, is the assemblage retaining ancestral character states of *Triantha* and should be divided at least into two species in future treatments. Before such a move, however, ample materials of *Tr. glutinosa* covering the whole range of distribution should be subjected to molecular analysis. The other two American species, *Tr. occidentalis* and *Tr. racemosa*, and the Japanese *Tr. japonica* are monophyletic with 59%, 98%, 91% support, respectively.

In *Triantha japonica*, a plant from Mt. Chokai (Yamagata), northern Honshu, with 94% support, diverged first from the remaining three plants, which are from Mt. Hakusan (Ishikawa), Mt. Shakadake (Ishikawa) and Mt. Mikuni (Shiga), all located in central Honshu. The relationships among *Tr. japonica* agree with the hypothesis of Fujii & Senni (2006), which emphasized that the mountains of central Honshu served as refugia for Japanese alpine plants during interglacial periods.

Concerning the distribution of *Tofieldia* and *Triantha*, an interesting common pattern is recognized: each major clade consists of one widely distributed boreal species, with several localized southern species. In the clade of *To. coccinea*, *To. nuda*, *To. divergens*, *To. yunnanensis* and *To. thibetica*, *To. coccinea* corresponds to the boreal species; in the clade of *To. okuboi*, *To. glabra* and *To. pusilla*, *To. pusilla* is the boreal species; in the clade of *Triantha* spp., it is *Tr. glutinosa*.

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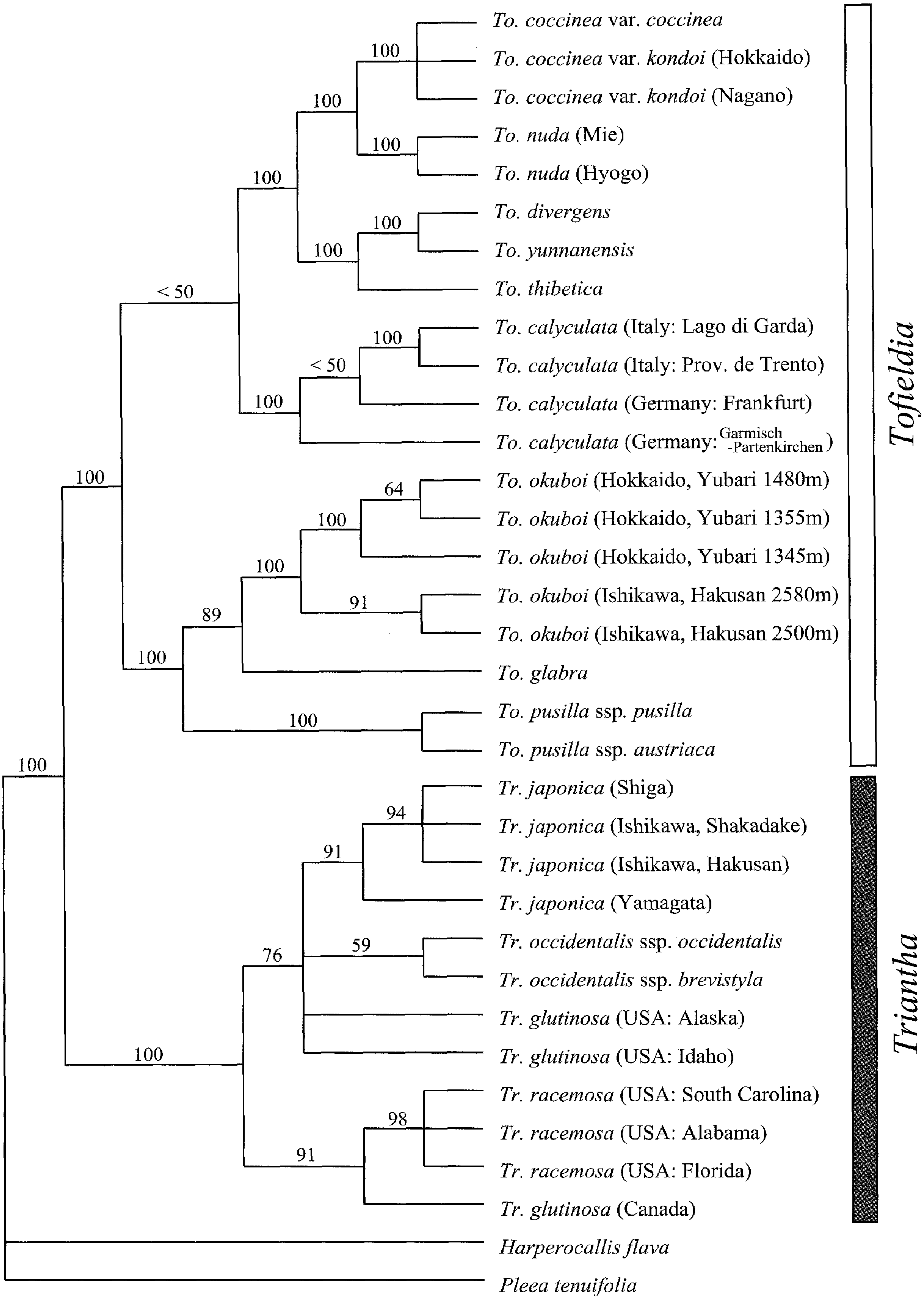
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To. coccinea var. coccinea
To. coccinea var. kondoi (Hokkaido)
To. coccinea var. kondoi (Nagano)
To. nuda (Mie)
To. nuda (Hyogo)
To. divergens
To. yunnanensis
To. thibetica
To. calyculata (Italy: Lago di Garda)
To. calyculata (Italy: Prov. de Trento)
To. calyculata (Germany: Frankfurt)
To. calyculata (Germany: Garmisch-Partenkirchen)
To. okuboi (Hokkaido, Yubari 1480m)
To. okuboi (Hokkaido, Yubari 1355m)
To. okuboi (Hokkaido, Yubari 1345m)
To. okuboi (Ishikawa, Hakusan 2580m)
To. okuboi (Ishikawa, Hakusan 2500m)
To. glabra
To. pusilla ssp. pusilla
To. pusilla ssp. austriaca
Tr. japonica (Shiga)
Tr. japonica (Ishikawa, Shakadake)
Tr. japonica (Ishikawa, Hakusan)
Tr. japonica (Yamagata)
Tr. occidentalis ssp. occidentalis
Tr. occidentalis ssp. brevistyla
Tr. glutinosa (USA: Alaska)
Tr. glutinosa (USA: Idaho)
Tr. racemosa (USA: South Carolina)
Tr. racemosa (USA: Alabama)
Tr. racemosa (USA: Florida)
Tr. glutinosa (Canada)
Harperocallis flava
Pleea tenuifolia
Tofieldia
Triantha

FIG. 1. Strict consensus of the 45 equally most parsimonious trees resulting from the combined analysis of plastid *trnK*, *trnL* and *trnL–F* and nuclear ITS DNA sequence data of *Tofieldia* and *Triantha*. Numbers above the branches indicate bootstrap values that are expressed as percentages of 1000 replicates.

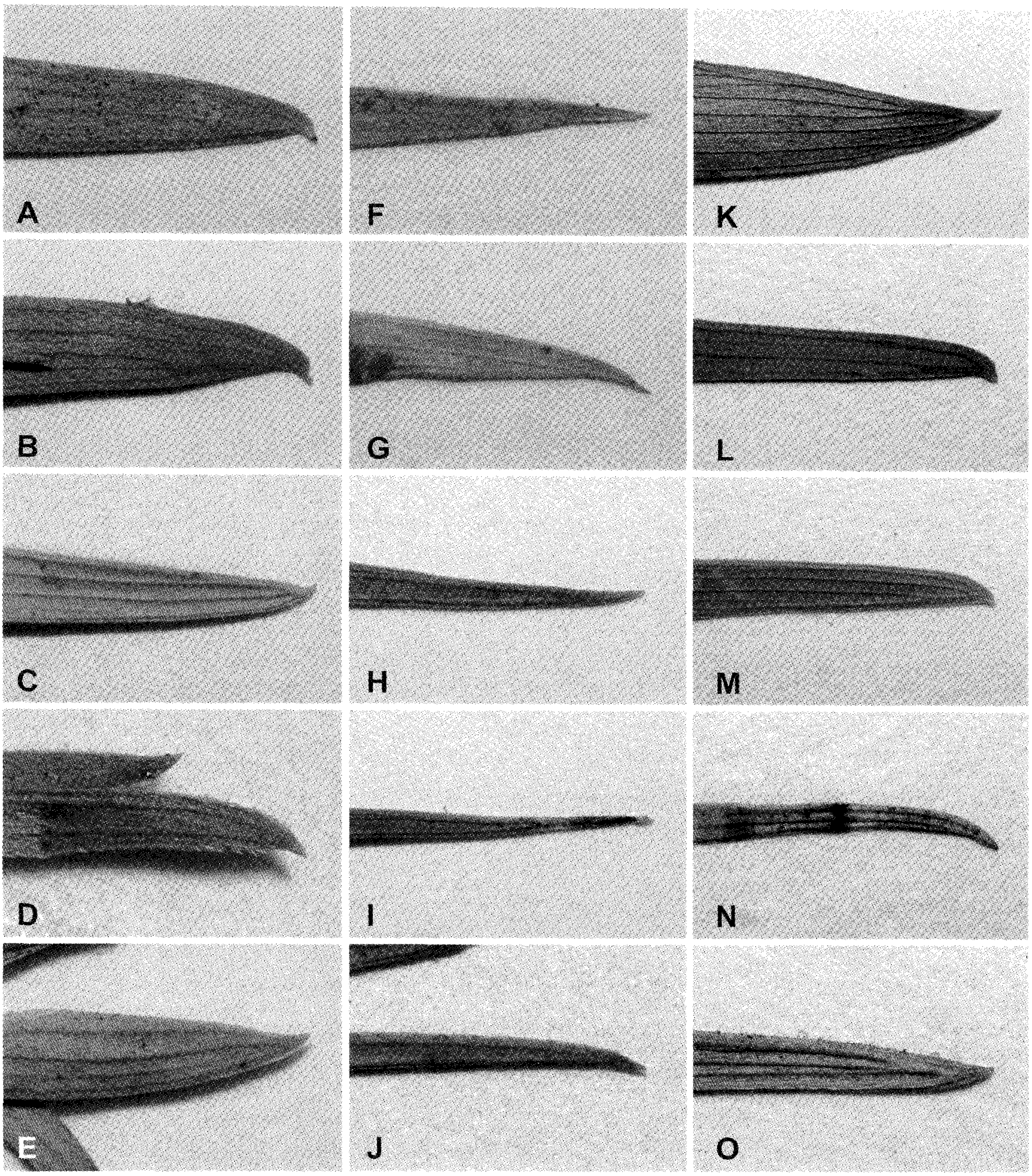

FIG. 2. Leaf apices of species of *Tofieldia* and species of *Triantha*. A: *To. okuboi* from Mt. Hakusan, 2500 m (*Tamura & Fuse 15521*, KYO). B: *To. okuboi* from Mt. Hakusan, 2580 m (*Tamura & Fuse 15519*, KYO). C: *To. glabra* (*Moldenke 113*, MO). D & E: *To. pusilla* subsp. *pusilla* (*Fredskild & Bay 3148*, MO). F & G: *To. coccinea* var. *coccinea* (*Parker & Lipkin 8884*, MO). H: *To. coccinea* var. *kondoi* (*Tamura 19499*, KYO). I: *To. nuda* (*Tamura & Fuse 15471*, KYO). J: *To. calyculata* (*Maquet 82/90*, MO). K: *Tr. japonica* (*Tamura & Fuse 15528*, KYO). L: *Tr. occidentalis* subsp. *brevistyla* (*Thompson 15079*, MO). M: *Tr. glutinosa* from USA (*Parker 5190*, MO). N: *Tr. glutinosa* from Canada (*Breteler 13914*, MO). O: *Tr. racemosa* (*Hill 23888*, MO).

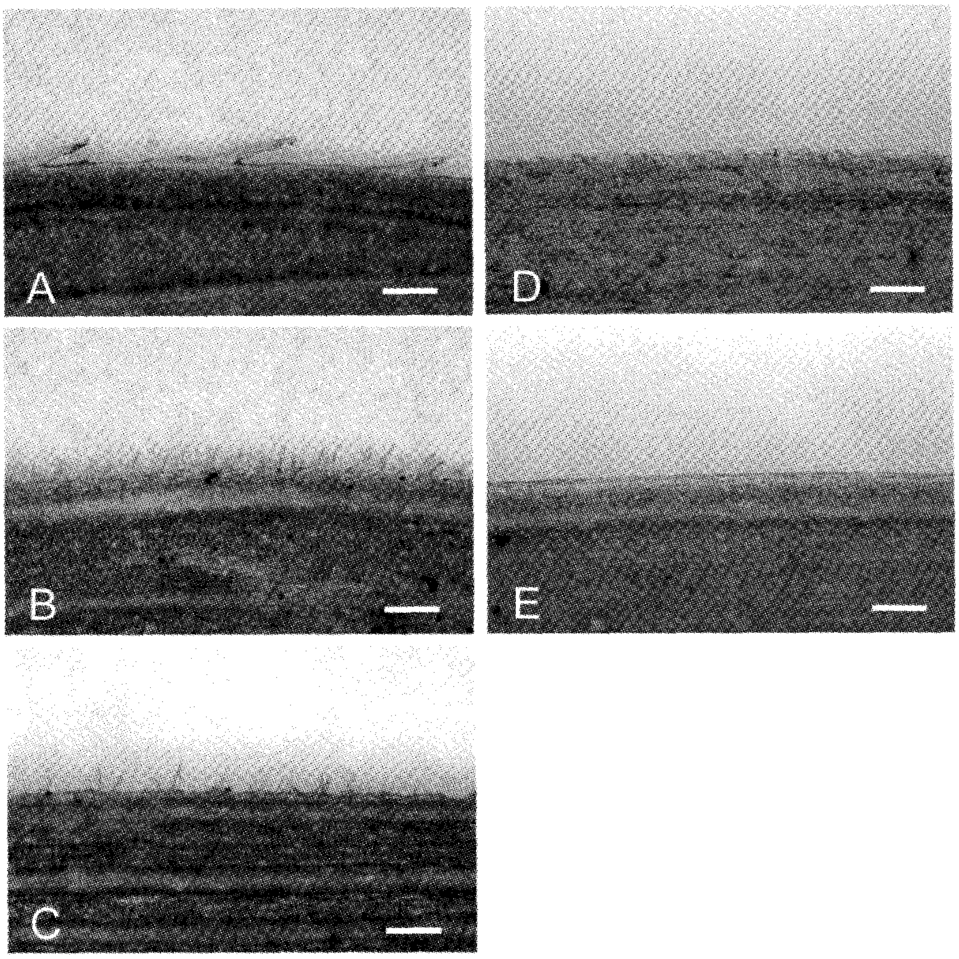

FIG. 3. Leaf margins of species of *Tofieldia*. A: *To. thibetica* (*Li 394*, MO) with long needle-like processes. B: *To. divergens* (*Bartholomew & al. 1470*, MO) with hair-like processes. C: *To. yunnanensis* (*Yuan 256*, MO) with hair-like processes. D: *To. coccinea* var. *kondoi* (*Tamura 19499*, KYO) with knobby processes. E: *To. nuda* (*Tamura & Fuse 15471*, KYO) without processes. Bar = 200 μm.

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